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13. ABSTRACT (Maximum 200 words)

The regulation of synaptic reactivity by protein kinase C and its substrates has been studied using the long-term potentation paradigm (LTP). We have studied the effects of protein kinase C activators and inhibitors on the durability of synaptic reactivity. The main conclusion to be drawn is that PKC is necessary but not sufficient for the enhanced durability. In combination with a neural signal, however, PKC demonstrates a profound synergism. Synergism is also observed in the analysis of metal ion regulation of PKC activity. Calcium and zinc interact in their effect on the enzyme in a bidirectional manner. Significant accomplishments made during this period were: determining the effect of inhibitors; the study of PKC activators (PDBu and oleate), metal ion regulation of PKC activity; and a second path for PKC activation.

14. SUBJECT TERMS

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Final Technical Report

1 October 1986 - 30 September 1989

Phosphoprotein Regulation of Synaptic Reactivity: Enhancement of a Molecular Gating Mechanism

Final Technical Report for AFOSR 88-0042

Submitted to:
Directorate of Life Sciences
Air Force Office of Scientific Research

Submitted by:
Dr. Aryeh Routtenberg
Cresap Neuroscience Laboratory
Northwestern University
Evanston, Illinois 60208

Final Technical/Scientific Report

0. Project Period The project period includes October 1, 1986 to September 30, 1989. The present report is being filed in July 1990.

1. Summary

The regulation of synaptic reactivity by protein kinase C and its substrate proteins has been studied using the long-term potentiation paradigm (LTP). We have studied the effects of protein kinase C activators and inhibitors on the durability of synaptic reactivity. The main conclusion to be drawn is that protein kinase C is necessary but not sufficient for the enhanced durability. In combination with a neural signal, however, PKC demonstrates a profound synergism. Synergism is also observed in the analysis of metal ion regulation of protein kinase C activity. Calcium and zinc interact in their effect on the enzyme in a bidirectinal manner (see below).

Significant accomplishments made during this period were:

a. Effect of inhibitors

We used three separate inhibitors of protein kinase C activity, polymyxin B, mellitin and H-7, each with a different mechanism of action. Application was made by micro-pressure ejection into the molecular layer of the dentate gyrus before or after LTP. The major result of this study was that inhibitors had no effect on the initiation of LTP but completely eliminated the enhanced response 10-15 min after its initiation. This provides strong support for our view that PKC plays a critical role in the maintanence but not the initiation of LTP.

b. Study of PKC activators (PDBu and oleate)

A crucial question in the analysis of the role of PKC in synaptic reactivity is the site of action of the compound. Indirect evidence suggested a synaptic site since PKC is found in high concentration there. To assess this view directly we compared application dosages required to facilitate synaptic reactivity duration in the dentate hilus, a nearby site, and the molecular layer of the dentate, 100 micra from the granule cells, precisely the point where perforant path terminals synapse. We have found that only 10-16% of the dosage is required when the application, iontophoretic or micro-pressure, is made at the synaptic zone. This provides strong support for the synaptic site of action of these protein kinase C activators.

c. Metal ion regulation of protein kinase C activity

Recent evidence describing the primary structure of protein kinase C by several laboratories indicates several different motifs: ATP-binding, Ca-binding, kinase domain, zinc "fingers". This suggested the possibility that both zinc as well as calcium might regulate protein kinase C activity.

Since we have recently discovered that protein kinase C can be activated in the absence of calcium it was now feasible to study the effects of zinc both in the presence and the absence of calcium. A novel mechanism for regulating protein kinase C activity was discovered in whichzinc ions, found in highest concentration in the hippocampus, enhance protein kinase C activity at low calcium levels. At higher levels of calcium, zinc inhibits. We propose a model of protein kinase C with a low and high calcium affinity binding sites and a distinct zinc binding site.

d. A second pathway for PKC activation

A major focus of our recent work has been on the novel protein kinase C (PKC) activators, the cis unsaturated fatty acids (CUFAs), which we have proposed represent an alternative route of activation of PKC since full activation can occur in the absence of calcium or phospholipid. We have zeroed in on this enzyme because there is a growing body of evidence that PKC regulates synaptic reactivity and learning and memory. To establish the cellular basis for this regulation we have studied ionic currents in cell lines and dissociated cells of the hippocampus where long-term potentiation paradigm (LTP) is demonstrated.

Since CUFAs are "physiological drugs" they offer the promise of a potentially safe methods for facilitating performance by enhancement of enzymes of the brain linked to memory and learning.

2. Statement of Work

We wished to know the answers to the following questions:

1. What regulates PKC activity? Is there another pathway, making use of another component of the phospholipid molecule, to effect transmembrane signalling?
   a- Are the effect of CUFAs specific for synapses? If so, are the effects specific for activated synapses?
   b- The effect of CUFAs is ultimately on the PKC-mediated phosphorylation of protein substrates. Using purified PKC and purified protein F1 we wish to know, What are the specific properties (time course, Km, calcium regulation, type of CUFA) that regulate this sequence of steps?
   c- What are the cellular consequences of CUFAs on ionic currents? Are these effects within the physiological range of CUFAs?
   d- What is the time course of PKC activity required for LTP?

3.Status of research

Significant accomplishments made during this period were:

a. Synapse-specific effects of PKC activators

1. Protein kinase C (PKC) stimulators, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or cis-unsaturated fatty acid (UFA), have been shown to prolong synaptic enhancement induced by long-term potentiation (LTP). This observation suggests a role for PKC in the biochemical mechanisms underlying maintained enhancement.
2. To determine if PKC stimulators prolong LTP by acting selectively at synapses given high frequency stimulation or by actions that are not synapse specific (e.g. increased postsynaptic excitability) we examined the effect of TPA or UFA on input-selective enhancement. Population EPSPs evoked in the same granule cell population by either the medial (MPP) or lateral (LPP) perforant path can be selectively enhanced leaving the other perforant path input which receives only low frequency stimulation as an internal control for PKC stimulator effects not specific to enhanced synapses.
3. Synapse specific effects were in fact observed as UFA or TPA selectively prolonged MPP enhancement following two trains of high frequency MPP stimulation, without affecting responses evoked by the LPP. A similar synapse selectivity of PKC stimulator action was seen following high frequency LPP stimulation.
4. These findings suggest that PKC stimulators prolong enhancement by acting specifically at high frequency

stimulated synapses. PKC stimulators do not appear to affect either postsynaptic neuron excitability or synapses given only low frequency stimulation. This provides further evidence that PKC acts synergistically with the consequences of repetitive synaptic activation to maintain enhancement.

b. Ionic currents regulated by CUFA mediated PKC activation

1. Activation of protein kinase C (PKC) by phorbol esters or diacylglycerols has been shown to modulate a number of ionic currents carried by Ca^{2+}, K^+, and Cl^-. Recently, it has been demonstrated that PKC may be activated by cis-fatty acids (c-FAs) in the absence of either phospholipid or Ca^{2+}. We wished to determine if this new class of PKC-activating compound would also modulate ionic currents, and if so, to determine the nature of that modulation. To this end, we applied the whole-cell patch clamp recording technique to N1E-115 neuroblastoma cells differentiated in 4% dimethylsulphoxide (DMSO).

2. Analysis of families of currents evoked under voltage clamp by depolarizing steps from a holding potential of -85 mV during application of 5 uM oleate (a c-FA) showed a 36% reduction of the peak inward current with no shift in either the peak or the reversal potential of the current-voltage (I-V) relation and no alteration of outward current.

3. As previous work has shown the inward current of this cell to be largely carried by Na^+, we sought to record the isolated Na^+ current by application of external Mg^{2+}, internal F^- and tetraethylammonium (TEA), and the replacement of internal K^+ with N-methylglucamine. The isolated Na^+ current recorded in this manner was completely and reversibly abolished by tetrodotoxin or removal of external Na^+, and was unaffected by application of external TEA.

4. Application of the c-FAs oleate, linoleate, and linolenate reversibly attenuated voltage-dependent Na^+ current with approximate ED50's of 2, 3, and 10 uM respectively. Elaidate (a trans-isomer of oleate) and stearate (a saturated fatty acid) which do not activate PKC, had no effect. Since cis-fatty acids are known to fluidize membranes, as well as to activate PKC, we sought to dissociate these functions by applying compounds that fluidize membranes but do not activate PKC: methyloleate and lysophosphatidylcholine. Neither compound affected Na^+ current when applied at concentrations of 1-50 uM.

5. In contrast to c-FAs, three classical PKC activators, phorbol-12,13-dibutyrate, phorbol-12,13,-diacetate, and 1,2-oleoylacetylglycerol (OAG) were found to have no effect on

the voltage-dependent Na^+ current when applied at 10nM-1uM (phorbol esters) or 1-150uM (OAG) for incubation periods up to 1 h.

6. The PKC inhibitors polymyxin B and H-7 were seen to block the attenuation of the Na^+ current by c-FA in a dose-dependent manner, with maximal inhibition occurring at doses of 50 and 10 uM, respectively. The cyclic nucleotide-dependent protein kinase inhibitor H-8 was much less effective in blocking the c-FA effect.

7. In another attempt to determine whether the c-FA effect was mediated by PKC, chronic (24 h) exposure to 1 uM phorbol ester was employed to down-regulate this enzyme. This treatment did not alter the baseline characteristcs of the isolated Na^+ current, but was effective in blocking the attenuation of Na^+ current produced by subsequent application of c-FA.

8. Taken together, these data suggest two broad classes of explanation. First, c-FA attenuation of the Na^+ current could be mediated in part through a non-PKC mechanism. The second explanation, which we favor, is that activation of the PKC family of enzymes by c-FAs and the classical PKC activators (phorbol esters, diacylglycerols) could result in different patterns of substrate phosphorylation such that c-FA activation of PKC produces attenuation of the Na^+ current in N1E-115 cells, while stimulation of PKC by classical PKC activators does not.

c. Substrate protein specific activation by CUFAs

CUFAs stimulated PKC mediated phosphorylation of purified protein F1, a synaptic plasticity-and growth-associated, neural-specific protein. Such phosphorylation occurred in the absence of phospholipids and diaglycerol. Maximal F1 phosphorylation occurred between 50-100 uM oleate (OA) or arachidonate (AA) in the presence of 200 uM Ca2+. Above 100 uM, however, an inhibitory effect was seen. Contrary to histone as a PKC substrate, the phosphorylation of protein F1 never reached maximal value (range: 0-400 uM OA and AA)in the absence of Ca2+; the highest phosphorylation level was 6-8 fold less than the highest level obtainable when 200 uM Ca2+ was added. These observations may be due to the differential stimulatory effects of CUFAs on various sub-types of PKC which, in turn, affect the phosphorylation kinetics of PKC substrates differently. Saturated fatty acids induced little stimulation of F1 phosphorylation either with or without Ca2+. The (+/-)Ca2+ ratios of F1 phosphorylation by PKC in the presence of 75 uM of various CUFAs were between 2.3 to 10.3. A comparison of the two-dimensional mappings of phospho-F1 showed no difference with regard to the

phosphorylation patterns between phospholipid and CUFA activation. **These findings suggest that CUFAs may represent a second messenger system for PKC activation which is distinct from phospholipid and diacylglycerol activation. Since CUFAs are less rapidly metabolized than diacylglycerol, we propose that CUFAs may provide for a more prolonged activation of PKC in plasma membrane.**

d. Synaptic reactivity: The necessity for PKC revealed by PKC inhibitors

Protein kinase C (PKC) activity is increased following hippocampal long-term potentiation (LTP, Akers et al., 1986). A similar increase in PKC activity is measured following the induction of a long-lasting potentiation with abbreviated high frequency stimulation in combination with PKC-activating phorbol esters (Colley et al., 1989). Since phorbol esters have no effect on the initial potentiaion produced with high frequency stimulation and PKC activity appears to be related to the persistence of LTP and not the magnitude of the initial change, we concluded that PKC regulates a post-initiation component of LTP. To define the time domain in which PKC activation is necessary for LTP, we studied the effect of PKC inhibitors, polymyxin B and H-7, micropressure ejected at different time points before and after the induction of LTP. LTP was produced in intact rats with high frequency stimulation of the perforant path and ejections were made in the molecular layer of the dentate gyrus. 1. Polymyxin B, which at lower doses is a selective inhibitor of PKC, had no effect on initial potentiation yet caused its decay to baseline within 2 hours when ejected 15 min before, 15 and 30 min, but not 60 min after high frequency stimulation. 2. Polymyxin B, at either low or high doses, was ineffective in blocking LTP persistence at time points greater than 30 min after high frequency stimulation. H-7 showed inhibitory effects comparable to low doses of polymyxin B on the persistence of LTP while also having no effect on initial potentiation. 3. In contrast to polymyxin B, higher doses of H-7 inhibited the potentiated response when delivered 240 min after high frequency stimulation. 4. A decrease in the in vitro phosphorylation of the PKC substrate protein F1 in animals that showed decay of LTP following inhibitor ejection suggests that polymyxin B and H-7 were inhibiting PKC in vivo. 5. We propose a model of LTP which consists of three separable phases: 1) initial potentiation (5-15 min) which does not require PKC activation; 2) a PKC-regulated persistence phase (15-120 min) related to modification of synaptic processes; 3) a second persistence phase (>120 min) which is dependent on PKC-regullated protein synthetic processes.

4. Articles published, accepted for publication and submitted.

Published

1. Nelson, R.B., Friedman, D.P., O'Neill, J.B., Mishkin, M., and Routtenberg, A. Gradients of PKC substrate phosphorylation in the primate visual cortical processing pathway peak in visual memory areas. Brain Research, 1987, 416, 387-392.
2. Akers, R.F. and Routtenberg, A. Calcium-promoted translocation of protein kinase C to synaptic membranes: relation to the phosphorylation of an endogenous substrate (protein F1) involved in synaptic plasticity. J. Neurosci., 1987, 7, 3976-3983.
3. Snipes, G.J., Chan, S., McGuire, C.B., Costello, B.R., Routtenberg, A., Norden, J.J., and Freeman, J.A. Evidence that GAP-43, a growth-related protein, and F1, a synaptic plasticity-associated protein, are identical. J. Neurosci., 1987, 7, 4066-4075.
4. Linden, D., Sheu, R.-S., Murakami, K., and Routtenberg, A. Cis fatty acid regulation of synaptic potentiation: Relation to phospholipase A2 and protein kinase C activation. J. Neurosci., 1987, 7, 4066-4075.
5. Murakami, K., Whiteley, M.K., and Routtenberg, A. Regulation of protein kinase C activity by cooperative interaction of Zn2+ and Ca2+. J. Biol. Chem., 1987, 262, 13902-13906.
6. Collier, T.J., Quirk, G.S., and Routtenberg, A. Separable roles of hippocampal granule cells in forgetting and pyramidal cells in remembering spatial information. Brain Research, 1987, 409, 316-328.
7. Lovinger, D.M. and Routtenberg, A. Protein F1 and protein kinase C may regulate the persistence of synaptic potentiation in the hippocampus. In. Ehrlich, Y.H., Berry, W., and Lennox, R. (Eds.). Molecular Mechanisms of Neuronal Responsitivity. Advances in Experimental Biology and Medicine, New York: Plenum 1987.
8. Ruth, R.E., Collier, T.J., and Routtenberg, A. Topography between the entorhinal cortex and the dentate septotemporal axis in rats: II. Lateral entorhinal projecting cells. J. Comp. Neurol., 1988, 270, 506-516.
9. Routtenberg, A. Phospholipid and fatty acid regulation of signal transduction at synapses: Potential role for protein kinase C in information storage. In: R.J. Wurtman, S. Corkin and J. Growden (Eds.). "Alzheimer's Disease: Advances in Basic Research and Therapies". Suppl. 24, New York: Springer-Verlag, 1987, pp. 239-246.
10. Lovinger, D.M. and Routtenberg, A. Synapse specific protein kinase C activation enhances maintenance of long-term potentiation in rat hippocampus. J. Physiol., Lond., 1988, 400, 321-333.

4. Articles published, accepted for publication and submitted.

Published

1. Nelson, R.B., Friedman, D.P., O'Neill, J.B., Mishkin, M., and Routtenberg, A. Gradients of PKC substrate phosphorylation in the primate visual cortical processing pathway peak in visual memory areas. Brain Research, 1987, 416, 387-392.
2. Akers, R.F. and Routtenberg, A. Calcium-promoted translocation of protein kinase C to synaptic membranes: relation to the phosphorylation of an endogenous substrate (protein F1) involved in synaptic plasticity. J. Neurosci., 1987, 7, 3976-3983.
3. Snipes, G.J., Chan, S., McGuire, C.B., Costello, B.R., Routtenberg, A., Norden, J.J., and Freeman, J.A. Evidence that GAP-43, a growth-related protein, and F1, a synaptic plasticity-associated protein, are identical. J. Neurosci., 1987, 7, 4066-4075.
4. Linden, D., Sheu, R.-S., Murakami, K., and Routtenberg, A. Cis fatty acid regulation of synaptic potentiation: Relation to phospholipase A2 and protein kinase C activation. J. Neurosci., 1987, 7, 4066-4075.
5. Murakami, K., Whiteley, M.K., and Routtenberg, A. Regulation of protein kinase C activity by cooperative interaction of Zn2+ and Ca2+. J. Biol. Chem., 1987, 262, 13902-13906.
6. Collier, T.J., Quirk, G.S., and Routtenberg, A. Separable roles of hippocampal granule cells in forgetting and pyramidal cells in remembering spatial information. Brain Research, 1987, 409, 316-328.
7. Lovinger, D.M. and Routtenberg, A. Protein F1 and protein kinase C may regulate the persistence of synaptic potentiation in the hippocampus. In: Ehrlich, Y.H., Berry, W., and Lennox, R. (Eds.). Molecular Mechanisms of Neuronal Responsitivity. Advances in Experimental Biology and Medicine, New York: Plenum 1987.
8. Ruth, R.E., Collier, T.J., and Routtenberg, A. Topography between the entorhinal cortex and the dentate septotemporal axis in rats: II. Lateral entorhinal projecting cells. J. Comp. Neurol., 1988, 270, 506-516.
9. Routtenberg, A. Phospholipid and fatty acid regulation of signal transduction at synapses: Potential role for protein kinase C in information storage. In: R.J. Wurtman, S. Corkin and J. Growden (Eds.). "Alzheimer's Disease: Advances in Basic Research and Therapies". Suppl. 24, New York: Springer-Verlag, 1987, pp. 239-246.
10. Lovinger, D.M. and Routtenberg, A. Synapse specific protein kinase C activation enhances maintenance of long-term potentiation in rat hippocampus. J. Physiol., Lond., 1988, 400, 321-333.

11. Rosenthal, A., Chan, S.Y., Henzel, W., Haskell, C., Kuang, W.-J., Chen, E., Wilcox, J.N., Ullrich, A., Goeddel, D.V., and Routtenberg, A. Primary structure and mRNA localization of protein F1, a growth-related protein kinase C substrate, associated with synaptic plasticity. EMBO J., 1987, 6, 3641-3646.
12. Lovinger, D.M., Wong, K.L., Murakami, K., and Routtenberg, A. Protein kinase C inhibitors eliminate hippocampal long-term potentiation. Brain Research, 1987, 436, 177-183.
13. Linden, D.J., Wong, K.L., Sheu, F.-S., and Routtenberg, A. NMDA receptor blockade prevents the increase in protein kinase C substrate (protein F1) phosphorylation produced by long-term potentiation. Brain Research, 1988, 458, 142-146.
14. Barnes, C.A., Mizumori, S.J.Y., Lovinger, D.M., Sheu, F.-S., Murakami, K., Chan, S.Y., Linden, D.J., Nelson, R.B., and Routtenberg, A. Selective decline in protein F1 phosphorylation in hippocampus of senescent rats. Neurobiology of Aging, 1988, 9, 393-398.
15. Nelson, R.B. and Routtenberg, A. Contrasting roles of a brain specific protein kinase C substrate: Has protein F1 evolved a new function in CNS of higher vertebrates? In: Proceedings of the IUPS Satellite Symposium on Cellular Mechanisms of Conditioning & Behavioral Plasticity (C. Woody, Ed.), 1988, pp 501-510.
16. Nelson, R. and Routtenberg, A. The protein F1/protein kinase C module and neurite growth: Potential utility in facilitating brain transplantation. In: Progress in Brain Research (J. Sladek and D. Gash, Eds.), Vol. 82, 1988.
17. Routtenberg, A. Protein kinase C and protein F1: Potential molecular mediators of lesion-induced synaptic plasticity recapitulate developmental plasticity. In: Post-lesion Neural Plasticity (H. Flohr, Ed.), Springer-Verlag, 1988.
18. Colley, P.A., and Routtenberg, A. Long-term potentiation and protein kinase C activity: dose-response analysis of phorbol esters. Brain Research, 1989, 495, 205-216.
19. Nelson, R.B., Linden, D.J., and Routtenberg, A. Phosphoproteins localized to presynaptic terminal linked to persistence of long-term potentiation: A quantitative analysis of two-dimensional gels. Brain Research, 1989, 497, 30-42.
20. Nelson, R.B., Hyman, C., Pfenninger, K.H., and Routtenberg, A. The two major phosphoproteins in growth cones are probably identical to two protein kinase C substrates correlated with persistence of long-term potentiation. J. Neurosci., 1989, 9, 381-389.
21. Routtenberg, A. Role of protein kinase C and protein F1 in presynaptic terminal growth leading to information storage. In: H. Rahmann (Ed.)

"Fundamentals of memory formation, "Progress in Zoology, 1989 Vol. 37, 283-295.
22. Routtenberg, A. Molecular basis of Hebb Synapse: Preserved mechanisms of axonal growth. In: M. Ito and Y. Nishizuka (Eds.), "Brain Signal Transduction and Memory," Academic Press, 1989, 213-227.
23. Linden, D.J., and Routtenberg, A. Cis-fatty acids, which activate protein kinase C, attenuate voltage-dependent Na+ current in mouse neuroblastoma cells. J. Physiol., 1989, 419, 95-119.
24. Wong, K.L., Murakami, K., and Routtenberg, A. Dietary cis fatty acids that increase protein F1 phosphorylation enhance spatial memory. Brain Research, 1990, 505, 302-305.
25. Linden, D.J. and Routtenberg, A. The role of protein kinase C in long-term potentiation. Brain Res. Revs., 1989, 14, 279-296.
26. Trommer, B.T. and Routtenberg, A. Long-term potentiation in intact infant rat hippocampus. Brain Research, 1990, 53, 288-290.

In press

1. Sheu, F.-S., Kasamatsu, T., and Routtenberg, A. Elevated protein kinase C activity and substrate (F1/GAP-43) phosphorylation in kitten visual cortex parallels critical period. Brain Res., 1990, in press.
2. Routtenberg, A. Long-term synaptic modification by protein kinase C: Regulation by activators, inhibitors, and NMDA receptors. Advances in Biochemical Pharmacology, G. Biggio, (Ed.), 1990, in press.
3. Routtenberg, A. Trans-synaptophobia. The Proceedings of the European Neuroscience Association Satellite Symposium on Excitatory Amino Acids and Neuronal Plasticity, Y. Ben Ari (Ed.), 1990, Plenum Press, in press.
4. Routtenberg, A. Action at a distance: The extracellular spread of chemicals in the nervous system. Volume Transmission in the Brain: New Aspects on Electrical and Chemical Communication, K. Fuxe (Ed.), 1990, Raven Press, in press.
5. Colley, P.A., Sheu, F.-S. and Routtenberg, A. Inhibition of protein kinase C blocks two components of LTP persistence leaving initial potentiation intact. J. Neuroscience, 1990, in press.

5. Personnel

A.

Name	Title	Dates of Service	% Effort
A. Routtenberg	Professor/PI	9/83-present	25%

S. Chan	Res. Neurobiologist	2/84-5/88	100%
K. Murakami**	Res. Neurobiologist	4/84-9/87	100%
P. Colley	Grad. Res. Asst.	7/83-present	50%
D. Linden	Grad. Res. Asst.	9/84-present	50%
D. Lovinger*	Grad. Res. Asst.	7/83-9/87	50%
R. Nelson*	Grad. Res. Asst.	7/83-9/87	50%
F. Sheu	Grad. Res. Asst.	9/85-present	50%
X. Xiang	Grad. Res. Asst.	1/88-present	25%
F. Cutting	Grad. Res. Asst.	9/88-present	25%
Y. Huang	Visiting Scientist	4/89-present	100%

* - Ph.D. awarded 6/87
** - Assistant Professor, University of Buffalo

Dr. Yan-You Huang from the Shanghai Institute of Physiology has joined my laboratory as a visiting scholar for at beginning April 1, 1989. Dr. Huang has had extensive research experience on the mechanism of LTP (working with Wigstrom and Gustaffson for several years). He is involved in a project concerning intracellular recording in hippocampal slices using CUFAs and will also carry out whole cell patch clamp studies on hippocampal cells to determine if the effects observed in neuroblastoma are present in non-transformed central nervous system nerve cells.

6. Coupling Activities

A. Spoken papers at meetings

1. Routtenberg, A. Invited speaker. Schmitt Symposium on Transplantation into the mammalin CNS. Rochester, N.Y., June 30-July 3, 1987.
2. Routtenberg, A. Invited speaker. "Lesion-induced neural plasticity." Bremen, Fed. Rep. of Germany, August 21-24, 1987.
3. Colley, P. and Routtenberg, A. Induction, enhancement and blockade of hippocampal synaptic potentiation: Dose-dependent effects of phorbol 12,13-dibutyrate (PDBu). Soc. Neurosci., 1987, 13, 1232.
4. Lovinger, D., Wong, K., Murakami, K., and Routtenberg, A. Protein kinase C (PKC) inhibitors prevent maintenance of hippocampal long-term potentiation (LTP). Soc. Neurosci., 1987, 13, 1233.
5. Linden, D.J., Sheu, F.-S., and Routtenberg, A. DL-aminophosphonovalerate (APV) blockade of hippocampal long-term potentiation (LTP) prevents an LTP-associated increase in protein F1 phosphorylation. Soc. Neurosci., 1987, 13, 1232.
6. Nelson, R.B., Linden, D.J., and Routtenberg, A. Two growth cone-enriched C-kinase substrates and two vesicle-associated phosphoproteins are directly

correlated with persistence of long-term potentiation: A quantitative analysis of two-dimensional gels. Soc. Neurosci., 1987, 13, 1233.
7. Sheu, F.-S., Murakami, K., and Routtenberg, A. Subcellular distribution of protein kinase C (PKC) in rat brain regions suggests that hippocampus has the highest capacity for kinase translocation, and that this capacity may be diurnally regulated. Soc. Neurosci., 1987, 13, 1009.
8. Murakami, K., Whiteley, M.K., and Routtenberg, A. Cooperative action of Zn(II) and Ca(II) in the regulation of protein kinase C activity from rat brain. Soc. Neurosci., 1987, 13, 1009.
9. Chan, S.Y., Haskell, C., and Routtenberg, A. The identification of protein F1 in mammalian nervous tissue using affinity-purified antibody against calf protein F1. Soc. Neurosci., 1987, 13, 1009.
10. Routtenberg, A. Invited speaker. Symposium on "Activity-dependent changes in synaptic efficacy in the vertebrate nervous system." London, England, November 5, 1987.
11. Routtenberg, A. Invited speaker. Symposium on "Review of Neurosciences." San Antonio, Texas, November 30-December 2, 1987.
12. Routtenberg, A. Invited speaker. Symposium on "Transmembrane Signaling and Cell Memory Processes." San Juan, Puerto Rico, December 11-13, 1987.
13. Routtenberg, A. Invited Session Organizer. "Perspectives on Alzheimer's Disease." Park City, Utah, January 9-13, 1988.
14. Routtenberg, A. Invited speaker. "Fundamentals of Memory Formation." Mainz, Fed. Rep. of Germany, October 27-29, 1988.
15. Linden, D., and Routtenberg, A. Cis-unsaturated fatty acids attenuate voltage-dependent sodium current in N1E neuroblastoma cells. Soc. Neuroscience, 1988, 14, 140.
16. Chan, S., Colley, P., and Routtenberg, A. LTP-induced protein F1 phosphorylation in vitro: Does it represent an increase or decrease in phosphate incorporation in vivo? Soc. Neurosci., 1988, 14, 18.
17. Routtenberg, A. Invited speaker. "Protein Kinase C and Neuronal Function." Kyoto, Japan, November 23-27, 1988.
18. Routtenberg, A. Invited speaker. "Preserved mechanisms of growth in synaptic plasticity." Island Neuroscience Conference, February 19-26, 1989.
19. Routtenberg, A. Invited speaker. "Molecular basis of Hebb synapse: preserved mechanisms of axonal growth." American Society for Neurochemistry, Chicago, Illinois, March 8, 1989.

20. Routtenberg, A. Invited speaker. "Protein kinase C regulation of synaptic plasticity. International Congress of Physiological Sciences, Helsinki, July, 1989. (Presented by D. Lovinger)
21. Routtenberg, A. Invited speaker. "Protein kinase C, synaptic plasticity and information storage." Fillerval meeting on "Excitatory amino acids and Neuronal Plasticity." Paris, France, August, 1989.
22. Routtenberg, A. Invited speaker. Wenner-Gren Symposium on Volume Transmission in the Brain: The Extracellular Fluid as a P' hway for Electrical and Chemical Communication. S ckholm, Sweden, September, 1989.
23. Routtenberg, A. Invited speaker. Society of Toxicology symposium on "Cellular and Molecular Mechanisms of Learning and Memory: Interactions and Neurotoxic Chemicals." Miami Beach, Florida, February 12, 1990. (Dr. H. Tilson)
24. Routtenberg, A. Co-organizer and speaker. Third International Phosphoprotein Meeting. Utrecht, The Netherlands, August, 1990. (With Dr. Willem Gispen)
25. Routtenberg, A. Invited speaker. "LTP: A Debate of Current Issues." Gif s/Yvette, France, October 3-5, 1990. (Dr. Michel Baudry)
26. Routtenberg, A. Invited speaker. Symposium on "Alzheimer's Disease: Status of Clinical and Basic Research." Mayo Clinic, Jacksonville, Florida, December 1 & 2, 1990. (Dr. Elliot Richelson)
27. Routtenberg, A. Invited speaker. "Zurich IV", the sixth meeting of the International Study Group on the Pharmacology of Memory Disorders Associated with Aging. Zurich, Switzerland, February 15-17, 1991.
28. Routtenberg, A. Invited speaker. 13th ISN Meeting Sydney, Australia, July 15-19, 1991. (Dr. R. Rodnight)

7. New Directions, Discoveries and Applications

In the last year we have found in an initial study that dietary CUFAs can enhance learning and memory related performance. The possible application to performance enhancement in humans and the continued exploration of the best conditions for achieving enhancement in animals suggests a promising direction for future research. The abstract of this initial study follows.

1. Abstract (From Wong, K.L., Murakami, K. and Routtenberg, A. Dietary cis fatty acids that increase protein F1 phosphorylation enhance spatial memory. Brain Research, 1990, 505, 302-305).

Cis fatty acids (c-FAs) activate protein kinase C (PKC) in the absence of calcium and phospholipid and promote the phosphorylation of protein F1 (aka GAP43). These c-FAs

facilitate long-term potentiation, a model of memory, and increase F1 phosphorylation. To determine if dietary c-FAs could alter memory itself as well as brain PKC/F1 metabolism, rats were maintained for 10 weeks on fatty acid diets enriched in oleate (OA; 20% olive oil, w/w), in oleate/linoleate (O/L; 20% corn oil), or in laurate/myristate (L/M; 20% hyrogenated coconut oil). In both the first two weeks of acquisition and in later achievement of criterion performance, the O/L diet group was superior to the other two groups in spatial memory performance. Hippocampal protein F1 in vitro phosphorylation in the O/L diet was significantly higher than in the other two groups in trained and nontrained animals. Animals that made fewer errors showed higher F1 phosphorylation (r=-0.70). Diet both increases brain PKC substrate phosphorylation and enhances maze learning, indicating the feasibility of enhancing a neurobiological learning mechanism by dietary means possibly ameliorating declines seen during normal and pathological aging.

2. Abstract (From: Society Neuroscience Abstract, 1990; SYNAPTIC PLASTICITY: MOLECULAR AND CELLULAR APPROACHES TO THE STUDY OF PROTEIN KINASE C (PKC) REGULATION OF PROTEIN F1/GAP-43 PHOSPHORYLATION. P. Meberg*, F-S. Sheu*, X. Xiang, Y.Y. Huang*, P. Colley*, B. Kapella*, E. Valcourt* and A. Routtenberg. Northwestern Univ., Evanston, IL 60208].

In situ hybridization of protein F1 mRNA demonstrates selective regional expression. Brainstem cells containing biogenic amines (e.g., locus coeruleus, dorsal raphe, substantia nigra-pars compacta) show extensive F1 mRNA expression, while other brainstem cells (e.g., red nucleus and pontine nucleus) and cholinergic cells (e.g., medial habenula) show little. The most striking selectivity is seen in adult hippocampus: pyramidal cells are heavily labeled while granule cells are at near background levels as quantified by image analysis (also, Rosenthal et al., EMBO J. 6:3641, 1987). Developmental analysis of F1 mRNA shows peak expression in pyramidal cells at 12 days, and in granule cells at 16 days; pyramidal cell F1 mRNA expression is 4- to 8-fold greater than the granule cells at all ages. Protein F1 is phosphorylated by PKC, whose subtype distribution, like F1, is regionally selective (Brandt et al., Cell 49:57, 1987). Protein F1 and beta-PKC mRNA are expressed at parallel levels (high-pyramidal; low-granule) in hippocampus, while gamma-PKC is expressed similarly in both cell types. Furthermore, beta-PKC, compared to gamma-PKC, preferentially phosphorylated either purified protein F1 or recombinant F1 made with protein-secreting bacteria. PKC regulates long-term potentiation (LTP; Routtenberg, Behav. Neural Biol. 44:186, 1985). Use of PKC inhibitors in the synaptic zone suggests PKC regulation of LTP persistence

not its initiation. The role of PKC in regulating plasticity in the cell body is currently under study using intracellular injection of PKC inhibitors into CA1 pyramidal cells in the hippocampal slice preparation. A protocol has been implemented to enable recording baseline activity with inhibitor-filled pipettes and ejection either before or after LTP. It is attractive to think that beta-PKC regulates persistence of LTP by phosphorylating presynaptic proteins.